

*Part of  
Paper #15  
5-30-03*

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re the Application of Timothy P. Croughan  
Serial No. 09/830,194

Examiner Kruse, D.

International Filing Date 05 November 1999

Group 1638

35 U.S.C. § 371 Date 23 April 2001

For: Herbicide Resistant Rice

Atty. File 98A9-US Croughan

MS Non-Fee Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**AFFIDAVIT OF INVENTOR TIMOTHY P. CROUGHAN**

**STATE OF LOUISIANA**

**PARISH OF ACADIA**

**Timothy P. Croughan**, being duly sworn, deposes and says:

1.

I am the inventor of the above-identified patent application. I make this Affidavit in support of that application.

---

**CERTIFICATE**

I hereby certify that this Affidavit is being deposited with the United States Postal Service as first class mail in an envelope addressed to: MS Non-Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 13, 2003.

  
John H. Runnels  
Registration No. 33,451  
May 13, 2003

2.

All experiments reported in the specification, and all experiments described in the present Affidavit were conducted by me, or were conducted under my supervision. (In addition to the experiments described below, several additional experiments were also run in parallel, experiments involving other resistant or non-resistant rice lines, or other herbicides, or both. In the interest of brevity, results from these parallel experiments are not given below, but can be supplied to the Office on request.)

3.

Pre-emergence herbicide applications were tested to identify the levels of two different herbicides that would completely inhibit germination for several rice lines. Seed of each line tested was germinated in a plastic disposable petri dish containing 8 mL of herbicide solution and a layer of Whatman No. 4 filter paper. The fungicide Vitavax 200 at a concentration of 0.5 mL/L was added to the incubation solutions to inhibit fungal growth. Untreated controls were incubated in solutions containing fungicide but no herbicide. Twenty seeds were placed in each of 3 replicate dishes per treatment, and were incubated at 25°C under 16 hour : 8 hour light/dark photoperiods at a fluorescent light intensity of 15 micro-Einstens per square meter per second. (One Einstein = 1 mole of photons.) Treatments were evaluated 11 days after incubation. The results of these pre-emergence experiments are shown in Table 1.

**Table 1. Herbicide Concentration (ppm) Needed to Completely Inhibit Germination**

	Cypress	ATCC 97523	ATCC PTA-904
Imazapic	1	10	60
Imazethapyr	2	10	80

4.

As shown in Table 1, line ATCC PTA-904 exhibited substantially higher resistance to pre-emergence applications of the herbicides imazapic and imazethapyr than did ATCC 97523 or "wild type" cultivar Cypress -- higher by a factor of about 6 to 8 in the former case, and by a factor of about 60 to 80 in the latter case. This very high level of pre-emergence resistance to imazapic and imazethapyr was surprising, and could not have been predicted from the characteristics of either Cypress or ATCC 97523.

5.

Post-emergence resistance has also been tested: in peat pots in a greenhouse, and on plants in the field. In the peat pot test, the tolerance of fourth generation ( $M_4$ ) plants to various post-emergence imidazolinone herbicide treatments was tested. Individual seedlings, in 3 replicate peat pots per treatment, were sprayed at the 2-3 leaf stage with 0X, 5X, 10X, 15X, and 20X herbicide treatments. Plants were rated 42 days after treatment. The values listed in Table 2 below were the highest tested rates that were tolerated with no visible injury, in some cases accompanied by a value in parentheses giving a higher rate at which the plants survived, but with injury. The post-emergence reaction of ATCC PTA-904 to the various herbicides tested was considerably different from that for ATCC 97523, and could have been predicted neither from the characteristics of ATCC 97523 nor from those of "wild type" Cypress.

**Table 2 -- Highest Rates of Post-Emergence Herbicide Treatment  
Tolerated without visible injury**

	Cypress	ATCC 97523 (93AS3510)	ATCC PTA-904 (PWC16)
<b>Imazethapyr</b>	All died at 5X	All died at 5X	10X (survived 15X)
<b>Imazamox</b>	All died at 5X	All died at 5X	All died at 5X
<b>Imazapyr</b>	All died at 5X	All died at 5X	10X
<b>Imazapic</b>	All died at 5X	All died at 5X	5X

Note: Except for imazamox, in each case a 10X application = 0.63 lb ai / A = 706 g ai / ha, and all other rates of application are proportional. For imazamox, a 10X application = 0.32 lb ai / A = 359 g ai / ha.

**6.**

Field tests were conducted to evaluate the herbicide-resistance of line PTA-904, the non-resistant rice variety Cypress, and the earlier resistant rice line ATCC 97523. All lines were planted in 1-meter rows, with two replications of each treatment. Post-emergence treatments of various herbicides were applied when the rice was at the 2-3 leaf stage of development. Herbicide applications were made with a backpack sprayer at a spray rate of 15 gallons per acre (163 liters per hectare). Evaluations of herbicide resistance were made as the plants reached the flowering stage, and were based on relative performance as compared to the non-treated control rows of the same lines. Results are shown in Table 3.

**Table 3 -- Post-Emergence Herbicide Tolerance in Field Trials or Greenhouse Trials.**  
**Entries give percent injury as compared with untreated controls of the same lines.**

	<b>Imazapic 0.075 lb ai/A + imazapyr 0.025 lb ai/A (=84 and 28 g ai / ha, respectively)</b>	<b>Rimsulfuron 0.025 lb ai/A (= 28 g ai / ha)</b>
<b>Cypress</b>	100% injury	100% injury
<b>ATCC 97523</b>	95% injury	90% injury
<b>ATCC PTA-904</b>	0% injury	100% injury

The herbicide treatments reported in Table 3 show that the herbicide resistance characteristics of PTA-904 differ markedly from those of ATCC 97523, and could have been predicted neither from the characteristics of ATCC 97523, nor from those of "wild type" Cypress.

All statements made in this Affidavit of my own knowledge are true. All statements made in this Affidavit on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified patent application or any patent issuing from that application.



\_\_\_\_\_  
Timothy P. Croughan

**SWORN TO AND SUBSCRIBED**

before me this 10th day  
of May, 2003.



\_\_\_\_\_  
NOTARY PUBLIC

John H. Runnels

[SEAL]



My Commission Expires at Death